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Magnesium nutrition of spinach (Spinacia oleracea L. var. inermis).

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MAGNESIUM NUTRITION OF SPINACH

(Spinacia oleracea L. var. inermis)

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INTRODUCTION

In recent years it has been observed that an extensive leaf chlorosis of spinach may follow periods of unusually heavy precipitation, especially when spinach is grown on light soils typical of the Eastern coastal region. Tentative field identification of the chlorosis as Mg^{++} deficiency and subsequent response of the chlorosis to foliar application of $MgSO_4$ has lead to this study of some of the conditions associated with the chlorosis. It is the aim and purpose of this thesis to investigate the conditions associated with the chlorosis and to study in detail some aspects of the magnesium nutrition of the spinach plant.

LITERATURE REVIEW

The term "spinach" as commonly used describes a vegetable of characteristic taste, color, and texture. However, this does not mean that the term is taxonomically pure because "spinach" does encompass genera from two families. The commonly grown market spinach is *Spinacia oleracea* of the Chenopodiaceae family while another herb, *Tetragonia expansa* or New Zealand spinach of the Aizoaceae family is sometimes found in gardens.

Spinacia the more familiar and older known genera is an annual dioecious herb of S. W. Asia. There are 3 or 4 species with *oleracea* being widely cultivated. In cool weather, the plant produces a large crown of smooth, triangular-ovate to hastate leaves alternately arranged. The flowers are unisexual. Pistillate flowers are axillary and usually clustered while staminate flowers are borne on terminal leafless spikes or panicles. The fruit is a utricle enclosed in a 2 - 4 spined capsule-like body (7) and is the reason for the term prickly-seeded spinach. Another species, inermis, lacks the spines and is termed smooth-seeded spinach (2). The first record of *Spinacia* edibility was found in Europe in 1351 although Albertus Magnus described *oleracea* in Germany before 1280. There are no notes of introduction into the United States but three varieties were known to occur in gardens in 1806 (13).

Tetragonia expansa is a native of New Zealand and Australia discovered in 1770 and now used as a spinach in various parts of the world except New Zealand (13). It is a vine-like annual herbaceous plant with vigorous growth providing a covering several feet thick. The leaves are somewhat succulent and deltoid in shape with a short petiole and are alternately arranged. The flowers are bisexual and are borne in the axils of leaves. There are usually 1 or 2 per axil. The fruit is top-shaped, 1/3 inch long, dry, hard, and thorny (2). The leaves, although much smaller than Spinacia oleracea, are similar in texture, color, and taste therefore leading to its common name. With this brief description of Tetragonia expansa it will not be mentioned again as the work described herein was performed entirely with Spinacia oleracea.

Spinach is a highly adaptable vegetable being grown in all parts of the United States. It occupies roles of varying importance depending upon the location. In most regions it plays only a small part in the vegetable production but is the most important crop in the eastern Oklahoma Arkansas River valley (22). In 1964, 209,000 tons were produced in the United States (45). In 1959, Massachusetts reported 61 growers with an average of 10.4 acres/grower and an average yield of 278 bu./acre (12). In 1965, spinach accounted for $270,000 of grower income and was the 24th most valuable vegetable crop in Massachusetts (46).

Mg^{++}, occupying 2 per cent of the earth's crust (4), has been known to be an essential element for plant growth for 60 or 70 years (18). Mg^{++} content of the soil, however, varies from only 0.05 per cent to 1.34 per cent in the United States (4). In normally cultivated soils there are four sources of Mg^{++}. The first of these are the Mg^{++} containing minerals such as olivine, serpentine, dolomite, biotite, chlorite, and others. The second source is any liming material which has been applied to the soil because all limestone contains some Mg^{++}, usually from 5 to 44 per cent as $MgCO_3$ (8). The third source of Mg^{++} is in commercial fertilizers, as impurities in low analysis fertilizers or in a fertilizer containing magnesium ammonium phosphate. The fourth source is from crop residues and manures decaying in the soil (9).

Whatever the source, the Mg^{++} must be present in the soil solution to be available for absorption by plants. This is accomplished by the slow decomposition caused by weathering or decay by microorganisms. The released Mg^{++} is then adsorbed by the surrounding clay particles or organic exchange materials (8). This exchangeable or available Mg^{++} is generally considered to be the most important as far as plant growth is concerned.

Mg^{++} is lost from the soil in several different ways. The first and major loss is through leaching by percolating waters (9). This loss may account for up to 80 per cent of the available Mg^{++} lost from the surface foot of soil in a year of normal precipitation (24). Other losses occur through erosion and crop removal and any excess of one of these can result in deficiency conditions.

Other conditions may also induce Mg^{++} deficiency. Beeson (6) has reported 125 different deficient areas all having coarse-textured soil. The ratios of the amounts of Ca^{++} to Mg^{++} and Mg^{++} to K^{+} are important. A high $Ca^{++}:Mg^{++}$ ratio or low $Mg^{++}:K^{+}$ ratio can induce Mg^{++} deficiency symptoms to appear in plants. An ideal ratio of 65 per cent Ca^{++}, 10 per cent Mg^{++}, 5 per cent K^{+}, and 20 per cent H^{+} is suggested by Bear and Toth (5).

Mg^{++} plays a number of roles in plant metabolism. Its most obvious function would be one in photosynthesis since it is the central atom in the chlorophyll molecule. However, it has not been shown that Mg^{++} is the active site in the molecule (14). It is the only metal atom in the molecule and comprises 2.7 per cent of the atomic weight although chlorophyll accounts for only 10 per cent of the total Mg^{++} in the leaf (18). Another role for Mg^{++} involves phosphate metabolism or transport. It has been demonstrated that plants grown on adequate Mg^{++} nutrition contain more phosphate than those on low Mg^{++} levels (37). More recent work suggests that instead of an actual transport of phosphate by Mg^{++} this increase may be due to Mg^{++} activated enzymes in phosphorus metabolism (31). T'so, et. al. (38) have found Mg^{++} associated with nucleic acids to a much greater extent than Ca^{++} and up to one half their combining capacity. Isolated enzyme studies have shown Mg^{++} to play an intregal part in group transfer reactions especially those involving phosphate. All this evidence suggests that Mg^{++} plays an important role in a variety of major metabolic processes.

It has already been mentioned that a balance of cations must exist in the soil solution. This balance was observed as early as 1900 in the case of Mg^{++} and Ca^{++} and the theory was established that a most desirable Ca:Mg ratio should exist for each crop. The subject of ion interrelationships has been reviewed by Jacob (18) where the explanation for the Ca^{++}, Mg^{++} antagonism is stated as one of mutual impairment of active absorption through competition for absorption carrier sites.

Shear, et. al. (33, 34) have expanded the cation balance theory to encompass the depressing effect of K^{+}, a major plant base, on the absorption of other cations. Laboratory work with yeast cells (10) and foliar analysis correlations (34) have definitely established that a high K^{+} level depresses the Mg^{++} content appreciably. Increased K^{+}

not only decreases the accumulation of Mg^{++} but also increases the level of requirement. Therefore, it is entirely possible that Mg^{++} deficiency in plants may be induced by an extremely high level of K^{+}

Another ion which acts very similarly to K^{+} in most cases is NH_4^{+}. It also has an antagonistic effect on Mg^{++} uptake and therefore the occurrence of Mg^{++} deficiency. This antagonism does not occur when nitrogen is applied as NO_3^{-}. In pot experiments (15), the effect of NH_4^{+} is complex resulting in acidic conditions and a direct inhibition of Mg^{++} uptake.

In addition to these antagonisms is the direct effect of pH. Mg^{++} deficiency is usually found on acid soils because they have lost their bases making the Mg^{++} compounds more soluble and more easily leached. Jacob (18) also suggests that the high mobility of the H^{+} ions may intensify the deficiency. Undoubtedly the former effect of the H^{+} ion is its major effect and this effect accounts for the finding that the Mg^{++} content of plants grown on acid soils is higher than plants grown on more basic ones (1).

It has been widely accepted and long known that plant nutrients can be leached from the soil. In 1804, DeSaussure proposed a theory that plant metabolites could be leached from foliage by aqueous solutions in similar fashion to soil leaching (43). Despite considerable experimentation and speculation which followed, the concept of foliar leaching was not fully developed until the last decade. The conclusive proof has been provided by a number of workers (39, 40, 41, 42, 44) using modern radioisotopic and chromatographic techniques. Today, therefore, the leaf assumes a much broader role beyond the classical concepts of photosynthesis and transpiration. Its modern role includes not only foliar leaching but also foliar absorption

The first report of the use of radioisotopes to determine the extent of foliar leaching appeared in 1956 by Long, et. al. (23). They reported that by growing young bean, sweetpotato, and poinsettia plants in labelled nutrient culture containing P^{32}, K^{42}, or Rb^{86} and leaching them under mist for 4 to 48 hrs. a significant reduction in root-absorbed K^{42} and Rb^{86} could be observed. Bean plants were the only ones to exhibit a reduction in P^{32}. The noted an increased amount of leaching in dark grown plants and with advanced plant maturity. Galactan was also identified as a component of the leachate.

Tukey, et. al. (39) used bean and squash plants to expand this work. They confirmed the work of Long, et. al. and found further that the amount of nutrient leached increased with duration of the leaching period, was greatest when the nutrient supply was maintained during leaching, that losses were greater from injured or nutritionally unbalanced leaves, and that nutrients leached from one plant species could be root-absorbed by another. Nutrients were classified as to their ease of leaching from very young leaves in a 24-hour period as follows: easily leached; Na^{24}, Mn^{54}, moderately leached; Ca^{45}, Mg^{28}, S^{35}, K^{42}, Sr^{90}-Y^{90}, and leached with difficulty; Fe^{55-59}, Zn^{65}, P^{32}, Cl^{36}. In addition to the inorganic plant nutrients they noted that carbohydrates were readily leached and that their losses paralleled light intensity (43, 44).

Corroborative studies performed by Tukey and Amling (42) used foliar leaching to explain the differences in nutrient levels between greenhouse and field grown plants. Levels of all nutrients except Cu and Fe were lower in field grown plants and this decrease in level could be attributed to the leaching action of rain or dew.

Mecklenburg and Tukey (26) found that the absorption rate of Ca^{45} was greater during leaching. This increase in absorption occurred whether the leached plants grew faster or slower than non-leached plants. They noted that 30 to 40 per cent of the recently root-absorbed Ca^{45} could be leached during a 4-day period and also that over an extended period an amount could be leached which was greater than that contained in the leaf.

Morgan and Tukey (30) characterized the organic components of the leachate. By fractionation and paper chromatography they were able to identify 21 amino acids and amides in each of 7 species. They identified 14 organic acids, including Kreb's cycle acids, in the anionic fraction and 4 free sugars from the neutral fraction. In addition, the neutral fraction contained other polysaccharides and carbohydrate material. Again, losses of carbohydrates were directly related to light intensity and independant of temperature suggesting that photosynthetic compounds were very easily leached.

Tukey (4) provides a good review of all material and outlines the factors which may influence the leaching of metabolites. These factors can be divided into two groups - internal and external. Internal factors concern the nature of the plant, i. e. species, wettability, and thickness of the cuticle; also the maturity, nutrient status, and physiological disorders play a part. External factors include leaching solution, light-darkness, duration and intensity of leaching, and the nutrition in the root medium both level and whether or not it is supplied to the plant during leaching.

The possibility of foliar absorption of plant nutrients has been accepted for many years. The first attempt at foliar nutrition was in the 17th century and western European grape growers have noted its beneficial effects for over a century (48). Beneficial responses vary from increased fresh weight to a more desirable color. Radioisotopes have stimulated new interest and a better understanding of foliar absorption (19) as well as foliar leaching.

The results of Teubner, et. al. (36) illustrate the efficiency of foliar sprays in a study of the utilization of radiophosphorus by several vegetable crops and tree fruits under field conditions. They found that foliar-applied P^{32} seemed to be used to a greater extent than did root absorbed P. They noted that total phosphorus in organs was a linear function of the number of sprays applied even though all soils contained adequate P.

Investigations designed to increase the understanding of foliar absorption have taken two paths. One is absorption by the intact leaf; the other, absorption by enzymatically isolated leaf parts.

Various techniques have been developed for absorption by the intact leaf. Jyung and Wittwer (19) used a leaf immersion technique; others have affixed glass wells to the leaf (21), or studied absorption from droplets (27, 29) or gas (17). Jyung and Wittwer have presented the most comprehensive work (19), determining that foliar absorption of Rb^+ and $H_2PO_4^-$ meets all criteria for an active uptake process. They have shown temperature, oxygen, energy, and pH dependance coupled with a sensitivity to metabolic inhibitors. Moorby (29) concurs with this finding in his study closely relating the uptake of Cs^{137} to a metabolic control and carbohydrate changes.

Middleton and Sanderson (27) take a slightly different view. They found independent absorption of anions and cations by intact plant leaves with Cs^{137} uptake being greater than I^-, $PO_4^{\equiv}$, or $SO_4^{=}$. In pointing out the similarity of their findings to the isolated cuticle studies they attribute a major role in uptake to permanent negative charges on the interior of the cuticle. It should be noted that this explanation accounts only for cuticle penetration and does not include any effect from absorption by mesophyll cells which probably was a dominant factor in the Jyung and Wittwer work.

The isolated cuticle work referred to above is that of Yamada, et. al. (49) where cuticles from stomatous and astomatous tissue were isolated and the ion penetration and ion binding were studied. They found that anions and cations penetrated cuticular membranes regardless of stomates and that differences in the direction of penetration were less pronounced when stomates were present; possibly because the cuticle within the stomate is thinner and more moist. The binding of ions on the inner surface was greater than the outer surface and this difference in binding capacity may cause the difference in penetration rates due to direction of passage. Yamada, in a later work (50), has shown that this system is not an active one but follows a curve very similar to that of a diffusion membrane.

There are many other reports of foliar absorption in the literature. Undoubtedly much interest is expressed in the potentially harmful isotopes arising from nuclear fallout. Middleton and Sanderson (27) included Sr^{89} in their studies and Hungate (17) has worked with I^{131}. These ions are especially useful because they are not normally found in plant cells. I^{131} studies have shown that its absorption was directly proportional to its concentration in the surrounding medium and similar in amount to that absorbed by a filter paper. They found that only 50 per cent of that deposited was removed by water, however, 90 per cent could be removed by other solvents. It was also noted that the I^{131} which did enter the plant was not translocated to other plant parts.

The role played by the stomates in foliar absorption of plant nutrients is obscure. One report has shown that they are not the main port of entry in foliar absorption (49). Also, it has been shown that in an intact leaf water may not penetrate the stomatal opening (27). However, workers have noted a definite correlation between stomatal frequency and absorption rate (20, 21). On the other hand, when studies concerning the degree of opening were performed (20) no correlation was observed. Therefore, it appears that foliar absorption of plant nutrients is related to stomatal frequency but not necessarily to stomatal aperture.

METHODS AND MATERIALS

Field Experiments

In the fall of 1965 a factorial experiment was designed to test the effects of soil pH and soil and foliar applications of $MgSO_4$ upon the cation content of spinach. The original pH of the soil used was 4.8-4.9. Hydrated lime was added at a rate equivalent to 2 tons of limestone per acre providing a second soil with a pH of 5.7 - 5.8. Results of the soil test performed by the University of Massachusetts Agricultural Experiment Station Soil Testing Laboratory appear in Table 1.

Table 1. - Soil test results

Description	pH	Ca	K	P	Mg
Unlimed	4.8-4.9	L	M	H	M
Limed	5.7-5.8	L	MH	H	H

On August 12, 1965, spinach var. Dixie Market[1] was seeded in 2 row, 20 ft. plots with 18 in. between the rows. Dixie Market was chosen because it is a commonly grown commercial variety. After germination the plots were cultivated and plants thinned to a 3 in. spacing within the rows. When noticeable foliage was apparent (2-3 weeks) the following treatments were applied:

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[1]Asgrow Seed Company, Milford, Conn.

1.0# Mg^{++}/acre foliar spray

1.5# Mg^{++}/acre foliar spray

40.0# Mg^{++}/acre soil treatment

80.0# Mg^{++}/acre soil treatment

40.0# Mg^{++} soil treat. + 1.0# Mg^{++}/acre foliar spray

80.0# Mg^{++} soil treat. + 1.0# Mg^{++}/acre foliar spray

Foliar sprays were applied at weekly intervals until marketable maturity when all plant material was harvested on October 17, 1965. Random leaf samples were taken for cation analysis.

Greenhouse Experiments

All greenhouse experiments were conducted in sand culture. Spinach was seeded directly in 4 or 5 gallon glazed clay crocks lined with polyethylene bags. The sand medium was a 1:1 mixture of fine and coarse pure quartz sand[2]. The crocks were subirrigated with nutrient solutions from 18 liter glass reservoirs. Solutions were raised by a pneumatic system and returned to the reservoirs by gravity; a separate reservoir being used for each crock. Nutrient solutions were replaced at 14 to 18 day intervals (25). In all cases the plants were thinned to 9 equally spaced plants per crock. Macronutrients were supplied from the nutrient solutions shown in Table 2. Minor nutrients were supplied in all treatments from the solution recommended by Hoagland and Arnon (16). Leaching was accomplished by suspending a perforated plastic lawn watering hose 2 ft. above the crocks and directing the fine streams of water downward onto the foliage. Two rows of crocks were leached simultaneously so that some stray water could be directed across the

[2]Supplied by Pennsylvania Glass Sand Corp., New York, N. Y.

bench providing even distribution and leaching. The leaching period was 96 consecutive hours in all cases. At marketable maturity or after the termination of leaching, plants were harvested. Fresh weights were recorded when pertinent and the leaves were segregated into age categories of young, immature, and mature.

Table 2. - Composition of nutrient solutions used in spinach nutrition studies

Nutrient Soln. No.	Mg^{++}	Ca^{++}	K^+	NO_3^-	$SO_4^=$	$H_2PO_4^-$	Cl^-
				meq./L.			
1	0.1	10.0	6.0	15.0	0.1	1.0	-
2	0.2	10.0	6.0	15.0	0.2	1.0	-
3	0.3	10.0	6.0	15.0	0.3	1.0	-
4	0.4	10.0	6.0	15.0	0.4	1.0	-
5	0.5	10.0	6.0	15.0	0.5	1.0	-
6	1.0	10.0	6.0	15.0	1.0	1.0	-
7	2.0	10.0	6.0	15.0	2.0	1.0	-
8	4.0	10.0	6.0	15.0	4.0	1.0	-
9	0.5	10.0	12.0	15.0	0.5	1.0	6.0
10	4.0	10.0	12.0	15.0	4.0	1.0	6.0

Laboratory Experiments

Experiments investigating the foliar penetration of Mg^{++} were conducted by methods similar to those used by Yamada (49). Cuticles were isolated and prepared as shown in Fig. 1. Leaf discs were cut from a washed mature spinach leaf with a large cork borer. Discs were placed in a solution of 2.0 per cent (w/v) pectinase[3], 0.1 per cent (w/v)

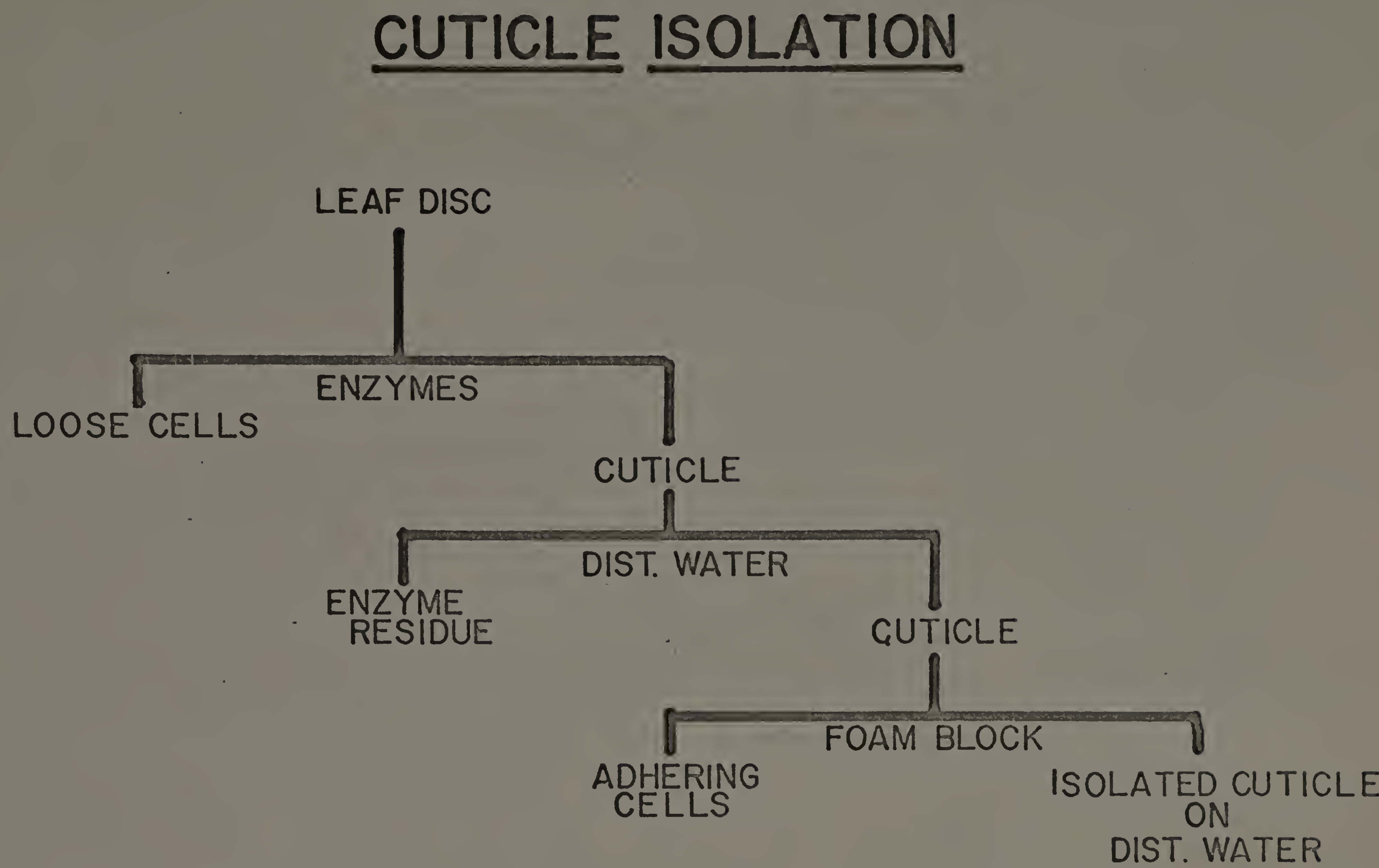

Fig.1 - Cuticle isolation procedure

cellulase[3], and 0.1 per cent (w/v) hemicellulase[3] in Walpole's acetate buffer (pH 3.8). Mild suction was applied to introduce the enzymes into the center of the disc (32). The incubation was continued for 24 hrs. at 37° C. in the dark. At this time the cuticles could be easily removed by gentle teasing with a curved glass rod and were transferred to a beaker of distilled water to remove the enzyme residue. The cuticles were removed from this rinse on a flat water-soaked foam block. While on the foam block the inner surface of the cuticle was wiped with a soft tissue to remove any remaining cellular debris. Cuticles were then floated on the surface of distilled water for attachment to the small tube in the test apparatus.

The apparatus for measuring the permeability of cuticular membranes is illustrated in Fig. 2. A 50 ml. cellulose nitrate centrifuge tube containing 35 ml. of 0.1 mM $MgSO_4$ was suspended in a constant temperature water bath. A smaller glass tube with a cuticle affixed to it was suspended in the $MgSO_4$ solution. One ml. of glass distilled water was pipetted into the smaller tube and the levels of solutions were matched to equalize hydrostatic pressure. A gentle aeration was employed to stir the solutions. Cuticles were affixed to the small tube by silicone rubber[4]. At the end of the test period the innermost solution was withdrawn for analysis.

<u>Analytical Methods</u>

Plant tissue to be analyzed for cation content was dried in a forced draft oven at 70° C. and ground in a Wiley mill to pass a 20

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[3]Nutritional Biochemicals Corporation, Cleveland, Ohio

[4]General Electric, Silicone Products Department, Waterford, N. Y.

CUTICLE ISOLATION APPARATUS

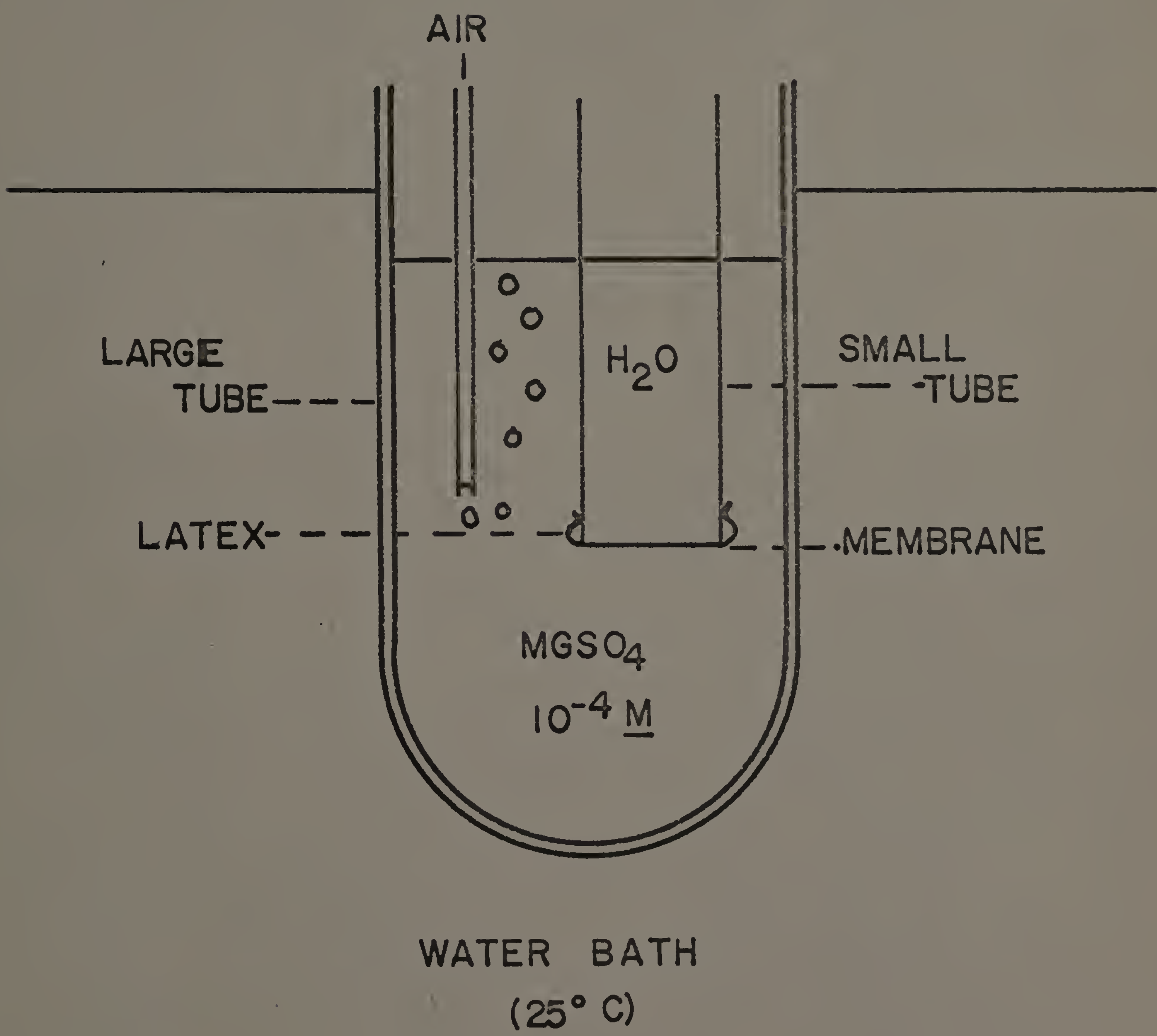

Fig. 2. - Cuticle isolation apparatus

mesh screen. A 100 mg. sample was placed in a 50 ml. Erlenmeyer flask and 5 ml. of concentrated HNO_3 added. The flask was heated until brown fumes cleared from it. The digestion was completed by adding 2 ml. of H_2O_2 dropwise. When the peroxide had boiled away the sample was washed quantitatively into a 100 ml. volumetric flask and made to volume with distilled water. This solution was diluted to the appropriate concentration range and an excess of $LaCl_3$ added to remove phosphate interference from the Ca^{++} assay. Determinations of Ca^{++} and Mg^{++} were performed with the aid of a Model 290 Perkin-Elmer Atomic Absorption Spectrophotometer. K^+ concentrations were determined with Model 214 of the same apparatus.

Statistical analysis of variance and Duncan's multiple range tests were performed by methods described by Steel and Torrie (35). A complete partitioning of treatment factors and interactions have been calculated in all cases but only the Duncan's multiple range tests are reported herein.

RESULTS

The results of a field experiment designed to increase the Mg^{++} content of spinach foliage by soil treatments and foliar sprays of $MgSO_4$ are presented in Table 3. At the low pH level the soil contained a small amount of available Mg^{++} and a direct response occurred between the amount of Mg^{++} applied in the treatments and the per cent Mg^{++} of the foliage. Significant increases in Mg^{++} content were obtained with a foliar spray of 1.5 # Mg^{++}/acre, all soil Mg^{++} treatments, and combinations of soil and foliar treatments. At the higher pH the only response occurred when a soil application of 80.0 # Mg^{++}/acre was combined with a foliar spray of 1.0 # Mg^{++}/acre. Concomitant with the rise in pH was a two-fold increase in the Mg^{++} content.

Ca^{++} percentages are inversely related to the Mg^{++} percentages. At the low pH a very high Ca^{++} content is noted and decreases with Mg^{++} application. At the higher pH the Ca^{++} content is consistantly lower and Mg^{++} applications have no effect. The K^+ content was not affected by either pH or Mg^{++} treatments

Table 3. - The effects of pH and $MgSO_4$ treatments on the cation content of spinach var. Dixie Market

Treatment	pH 4.8	pH 5.8
	per cent Mg^{++}	
Control	.41a	.91 cd
1.0# Mg^{++}/acre foliar	.44a	.99 d
1.5# Mg^{++}/acre foliar	.53ab	1.04 d
40.0# Mg^{++}/acre soil	.69 bc	1.05 d
80.0# Mg^{++}/acre soil	.89 cd	.98 d
40.0# soil + 1.0# foliar	.88 cd	1.01 d
80.0# soil + 1.0# foliar	1.01 d	1.32 e
	per cent Ca^{++}	
Control	1.03 d	.67abc
1.0# Mg^{++}/acre foliar	.83 cd	.71 bc
1.5# Mg^{++}/acre foliar	.67abc	.72 bc
40.0# Mg^{++}/acre soil	.61abc	.69abc
80.0# Mg^{++}/acre soil	.56ab	.75 bc
40.0# soil + 1.0# foliar	.69abc	.64abc
80.0# soil + 1.0# foliar	.47a	.77 bc
	per cent K^{+}	
Control	3.53a	3.95a
1.0# Mg^{++}/acre foliar	3.54a	3.90a
1.5# Mg^{++}/acre foliar	4.50a	4.61a
40.0# Mg^{++}/acre soil	3.92a	3.95a
80.0# Mg^{++}/acre soil	3.53a	4.32a
40.0# soil + 1.0# foliar	4.04a	4.10a
80.0# soil + 1.0# foliar	3.24a	3.92a

Means within a cation not followed be the same letter are significantly different at the 5 per cent level.

Table 4 contains the results of a sampling experiment performed in the greenhouse. Significant differences in cation content were observed between lamina and petioles as shown in Table 4a. Petioles had a lower Mg^{++} and Ca^{++} content but a higher percentage of K^{+}. When samples contained both lamina and petioles the percentages of all cations were between values of the lamina and petioles when taken separately but not significantly different from the lamina samples. The data in Table 4b show that increasing the Mg^{++} level of the nutrient solution from 0.1 to 0.5 meq/L has no significant effect on the cation content however a further increase to 2.0 meq/L significantly increases the Mg^{++} content and depresses the Ca^{++} content. Maturity is accompanied by an increase in the percentages of all cations as presented in Table 4c. The results in Table 4d show the significant interaction of Mg^{++} level and age since increasing either one increases the Mg^{++} content. At the lower Mg^{++} levels the tissue age has no effect and in young tissue the effect of increasing Mg^{++} levels is not so great as in older tissue. The data in Table 4e indicate that in young tissue the Mg^{++} level is uniform regardless of sampling position, while the Mg^{++} content of mature petioles is significantly reduced. The data in Table 4f show that the Mg^{++} content of the lamina, petiole, and lamina + petiole samples was not different at the low Mg^{++} level. At the higher Mg^{++} level the Mg^{++} content of the petioles was significantly less than the Mg^{++} content of the lamina or lamina + petiole samples.

Table 4. - The effects of Mg^{++}level, age of tissue, and tissue sample on cation content of spinach var. Dixie Market

Mg^{++} Level	Lamina		Petioles		Lamina+Petioles	
	Young	Mature	Young	Mature	Young	Mature
(meq/L)			per cent Mg^{++}			
0.1	.11a	.14a	.08a	.08a	.08a	.09a
0.5	.16ab	.22 bc	.16ab	.16ab	.13a	.15ab
2.0	.25 c	.93 e	.28 c	.48 d	.28 c	.84 f
			per cent Ca^{++}			
0.1	1.86 c	4.05 f	.58a	1.08ab	1.53 bc	3.39 def
0.5	1.37abc	5.61 g	.91ab	1.25abc	1.11abc	3.86 ef
2.0	1.06ab	3.14 de	.70a	.80ab	1.11abc	2.75 d
			per cent K^{+}			
0.1	6.00ab	10.27 e	8.26 c	11.45 f	6.99 b	9.37 cde
0.5	5.52a	8.81 cd	8.33 c	9.58 de	6.16ab	8.61 cd
2.0	5.93ab	8.95 cd	8.19 c	12.36 f	6.33ab	9.58 de

Means within a cation not followed by the same letter are significantly different at the 5 per cent level.

Table 4a. - The variation of cation content with tissue sample in spinach

Sample	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Lamina	.30 b	2.85 b	7.58a
Petioles	.20a	.89a	9.69 b
Lamina + Petioles	.26ab	2.29 b	7.84a

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 4b. - The effect of Mg^{++} level on cation content of spinach

Mg^{++} Level	Mg^{++}	Ca^{++}	K^{+}
(meq/L)		per cent	
0.1	.10a	2.08ab	8.72 b
0.5	.16a	2.35 b	7.83a
2.0	.51 b	1.59a	8.55ab

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 4c. - The effect of age of tissue on cation content of spinach

Age	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Young	.17a	1.14a	6.86a
Mature	.34 b	2.88 b	9.89 b

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 4d. - The effect of Mg^{++} level and age of tissue on the Mg^{++} content of spinach

Mg^{++} Level	Young	Mature
(meq/L)	per cent Mg^{++}	
0.1	.09a	.10a
0.5	.14ab	.18ab
2.0	.27 b	.75 c

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 4e. - The effect of tissue sample and age of tissue on Mg^{++} content of spinach

Sample	Young	Mature
	per cent Mg^{++}	
Lamina	.17a	.41 b
Petioles	.17a	.24a
Lamina + Petioles	.16a	.36 b

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 4f. - The effect of Mg^{++} level and tissue sample upon the Mg^{++} content of spinach

Mg^{++} Level	Lamina	Petioles	Lamina+Petioles
(meq/L)		per cent Mg^{++}	
0.1	.12a	.08a	.09a
0.5	.19ab	.16a	.14a
2.0	.59 d	.38 bc	.56 cd

Means not followed by the same letter are significantly different at the 5 per cent level.

An experiment with very low levels of Mg^{++} was designed to establish a critical level of Mg^{++} within a variety. A visual yellowing of spinach foliage was noted when the plant, at marketable maturity, was placed in a nutrient solution containing 0.3 meq/L Mg^{++} or lower. After several days, leaves from the deficient plants developed a characteristic chlorosis as shown in Figure 3. The cation content of spinach cultured at very low Mg^{++} levels is shown in Table 5 with details in subsequent tables. The results shown in Table 5a indicate no significant increase in Mg^{++} content at the lower Mg^{++} levels. Ca^{++} percentage was not affected while the K^{+} percentage declined slightly with increasing Mg^{++} levels. In Table 5b it can be seen that Mg^{++}, Ca^{++}, and K^{+} all tend to accumulate in the older tissue; this effect is most pronounced in the case of Ca^{++}. There was no significant interaction between age and Mg^{++} level in this experiment.

Figure 3. - Examples of slight and severe Mg^{++} deficiency in spinach var. Dixie Market

Table 5. - The influence of low Mg^{++} levels on the cation content of spinach var. Dixie Market

Mg^{++} Level	Young	Mature
(meq/L)	per cent Mg^{++}	
0.1	.11a	.14ab
0.2	.11a	.11a
0.3	.12ab	.16ab
0.4	.13ab	.17 b
0.5	.14ab	.22 c
	per cent Ca^{++}	
0.1	1.86a	4.05 b
0.2	1.92a	4.44 b
0.3	1.58a	4.19 b
0.4	1.50a	4.22 b
0.5	1.33a	5.61 c
	per cent K^{+}	
0.1	5.60a	10.27 b
0.2	5.41a	9.37 b
0.3	5.41a	9.82 b
0.4	5.34a	8.60 b
0.5	5.52a	8.81 b

Means within a cation not followed by the same letter are significantly different at the 5 per cent level.

Table 5a. - The effects of low Mg^{++} levels on cation content in spinach

Mg^{++} Level	Mg^{++}	Ca^{++}	K^{+}
(meq/L)		per cent	
0.1	.12ab	2.96a	8.14 b
0.2	.11a	3.18a	7.39ab
0.3	.14ab	2.89a	7.62ab
0.4	.15 bc	2.86a	6.97a
0.5	.18 c	3.47a	7.16a

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 5b. - The effect of tissue age on cation content of spinach

Age	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Young	.12a	1.64a	5.54a
Mature	.16 b	4.50 b	9.38 b

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

The results of an experiment designed to investigate the ability of different spinach cultivars to accumulate Mg^{++} is shown in Table 6. The data in Table 6a indicate no differences in cation content due to variety with the exception of Blight Resistant Savoy having a low K^{+} content. Increasing the Mg^{++} level increased the Mg^{++} content while decreasing the Ca^{++} percentage, as shown in Table 6b, but did not affect the K^{+} content. Mature tissue contained a greater amount of all cations (Table 6c) in all varieties (Table 6f), however, in the case of Mg^{++} this increase was only apparent at the high (4.0 meq/L) Mg^{++} level

(Table 6d). When considering the Mg^{++} level and cultivar interaction (Table 6e) it is apparent that at the high Mg^{++} levels Dixie Market and Savoy Supreme accumulate less Mg^{++} than Virginia Savoy and Blight Resistant Savoy. No differences were noted at the low Mg^{++} level.

Table 6. - The effects of Mg^{++} level and age of tissue on the cation content of spinach varieties

	0.5 meq/L Mg^{++}		4.0 meq/L Mg^{++}	
Cultivar	Young	Mature	Young	Mature
		per cent Mg^{++}		
Dixie Market	.14a	.21ab	.31abc	1.14 d
Virginia Savoy	.14a	.22ab	.43 c	1.16 d
Savoy Supreme	.14a	.20ab	.30abc	1.19 d
Blight Resistant Savoy	.14a	.18ab	.35 bc	1.38 e
		per cent Ca^{++}		
Dixie Market	.61a	3.31 d	.36a	1.75 b
Virginia Savoy	.47a	2.47 c	.58a	1.83 b
Savoy Supreme	.55a	2.56 c	.41a	1.89 b
Blight Resistant Savoy	.47a	2.92 cd	.42a	2.92 cd
		per cent K^{+}		
Dixie Market	6.48a	10.51 de	7.18a	10.97 d
Virginia Savoy	7.20a	10.72 de	7.53ab	9.78 cd
Savoy Supreme	6.91a	10.69 de	7.29a	11.45 e
Blight Resistant Savoy	6.67a	9.16 c	6.96a	8.74 bc

Means within a cation not followed by the same letter are significantly different at the 5 per cent level.

Table 6a. - The effect of cultivar on the cation content of spinach

Cultivar	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Dixie Market	.15a	1.51a	8.78 b
Virginia Savoy	.16a	1.34a	8.81 b
Savoy Supreme	.15a	1.35a	9.08 b
Blight Resistant Savoy	.17a	1.60a	7.88a

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 6b. - The effect of Mg^{++} level on the cation content of spinach

Mg^{++} Level	Mg^{++}	Ca^{++}	K^{+}
(meq/L)		per cent	
0.5	.17a	1.67a	8.54a
4.0	.78 b	1.23 b	8.74a

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 6c. - The effect of age of tissue on the cation content of spinach

Age	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Young	.25a	.49a	7.03a
Mature	.71 b	1.99 b	10.25 b

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 6d. - The effect of Mg^{++} level and age on the Mg^{++} content of spinach

Mg^{++} Level	Young	Mature
(meq/L)	per cent Mg^{++}	
0.5	.14a	.20a
4.0	.35 b	1.22 c

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 6e. - The effect of Mg^{++} level and variety on the Mg^{++} content of spinach

Cultivar	0.5 meq/L Mg^{++}	4.0 meq/L Mg^{++}
Dixie Market	.18a	.73 b
Virginia Savoy	.18a	.80 bc
Savoy Supreme	.17a	.75 b
Blight Resistant Savoy	.16a	.86 c

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 6f. - The effect of variety and age of tissue on the Mg^{++} content of spinach varieties

Cultivar	Young	Mature
	per cent Mg^{++}	
Dixie Market	.23a	.68 b
Virginia Savoy	.29a	.69 b
Savoy Supreme	.22a	.70 b
Blight Resistant Savoy	.25a	.78 b

Means not followed by the same letter are significantly different at the 5 per cent level.

The cation analysis of an experiment designed to investigate the effects of Mg^{++} level, age of tissue, and foliar leaching on the cation content of spinach var. Savoy Supreme is presented in Table 7. Increasing the Mg^{++} supply increased the Mg^{++} content and depressed the Ca^{++} content while the K^{+} remained largely unchanged (Table 7a). There was an overall increase in Mg^{++}, Ca^{++}, and K^{+} with increasing maturity (Table 7b). When considering the significant interaction between Mg^{++} level and age (Table 7d), it becomes apparent that mature tissue is responsible for the increase in Mg^{++} content associated with increasing Mg^{++} levels. Young tissue shows no significant response to increased Mg^{++}, immature tissue is significantly affected at higher levels, whereas mature tissue reflects every increment of change. A detailed analysis (Table 7c) of the relationship between cation content of spinach as related to foliar leaching indicates that significant quantities of Mg^{++}, Ca^{++}, and K^{+} were leached from the tissue.

Table 7. - The effects of Mg^{++} level, age of tissue, and foliar leaching on the cation composition of spinach var. Savoy Supreme

	Control			Leached		
Mg^{++} Level	Mature	Immature	Young	Mature	Immature	Young
(meq/L)			per cent Mg^{++}			
0.5	.41 de	.20ab	.16a	.29 bc	.17a	.16a
1.0	.63 f	.35 c	.20a	.43 e	.28 bc	.18a
2.0	.93 h	.41 d	.23ab	.77 g	.33 cd	.18a
4.0	1.38 i	.57 f	.30 b	1.03 h	.48 e	.21ab
			per cent Ca^{++}			
0.5	3.12 h	.77 cd	.23	1.94 g	.79 d	.17ab
1.0	1.77 fg	.84 d	.33abc	1.29 e	1.10 de	.23ab
2.0	1.81 fg	.79 d	.25ab	1.36 e	.64 bcd	.19ab
4.0	1.94 g	.65 bcd	.19ab	1.42 ef	.64 bcd	.15a
			per cent K^{+}			
0.5	11.29 fgh	8.25 de	7.04 b	10.41 f	7.80 bcd	5.34a
1.0	12.08 h	9.11 e	7.12 b	10.75 f	8.10 d	6.15a
2.0	11.89 gh	8.64 de	7.13 b	10.62 f	7.80 bcd	6.02a
4.0	11.11 fg	8.51 de	7.28 bc	10.56 f	8.20 de	6.18a

Means within a cation not followed by the same letter are significantly different at the 5 per cent level.

Table 7a. - The effect of Mg^{++} level on the cation content of spinach

Mg^{++} Level	Mg^{++}	Ca^{++}	K^{+}
(meq/L)		per cent	
0.5	.23a	1.17 b	8.36a
1.0	.34 b	.93a	8.88 b
2.0	.47 c	.92a	8.63ab
4.0	.66 d	.83a	8.65ab

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 7b. - The effect of age of tissue on the cation content of spinach

Age	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Young	.20a	.15a	6.54a
Immature	.32 b	.78 b	8.29 b
Mature	.73 c	1.89 c	11.06 c

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 7c. - The effect of foliar leaching on the cation content of spinach

Treatment	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Control	.48 b	1.10 b	9.10 b
Leached	.37a	.83a	8.16a

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 7d. - The effects of Mg^{++} level and age on the Mg^{++} content of spinach

Mg^{++} Level	Mature	Immature	Young
(meq/L)		per cent Mg^{++}	
0.5	.35ab	.19a	.16a
1.0	.53 b	.32ab	.19a
2.0	.85 c	.37ab	.20a
4.0	1.20 d	.53 b	.26a

Means not followed by the same letter are significantly different at the 5 per cent level.

The results of an experiment investigating the effects of Mg^{++} level, K^+ level, age of tissue, and foliar leaching on the cation content of Dixie Market spinach are presented in Table 8. Increasing the Mg^{++} level increases the Mg^{++} content of both young and mature tissues (Table 8e) while significantly depressing the Ca^{++} percentage although not affecting the K^+ content (Table 8a). Increasing the K^+ level of the nutrient solution produced an increase in the K^+ content and depressed the Ca^{++} content but did not influence the Mg^{++} percentage (Table 8b). Maturity increased the percentage of all cations (Table 8c). In this experiment, unlike the previous one, foliar leaching produced a decrease in only Mg^{++} and K^+ while the Ca^{++} content was unchanged (Table 8d). The interaction between K^+ level and leaching on the Mg^{++} content of spinach is shown in Table 8f. The depression of Mg^{++} content caused by increasing the K^+ level is not significantly different from the reduction caused by foliar leaching; foliar leaching did not decrease the Mg^{++} content at the high K^+ level. K^+ was effective only in decreasing the Mg^{++} content at the higher Mg^{++} concentrations (Table 8g).

Table 8. - The effects of Mg^{++} level, K^{+} level, age of tissue, and foliar leaching on the cation content of spinach var. Dixie Market

Nutrient Soln.	Control Young	Control Mature	Leached Young	Leached Mature
(meq/L)		per cent Mg^{++}		
0.5 Mg^{++} & 6.0 K^{+}	.29ab	.44 bc	.22a	.32ab
4.0 Mg^{++} & 6.0 K^{+}	.71 e	1.69 h	.46 bc	1.47 fg
0.5 Mg^{++} & 12.0 K^{+}	.27ab	.43 bc	.21a	.35abc
4.0 Mg^{++} & 12.0 K^{+}	.52 cd	1.46 f	.67 de	1.61 fgh
		per cent Ca^{++}		
0.5 Mg^{++} & 6.0 K^{+}	1.05 bcde	2.03 g	.70abc	2.75 h
4.0 Mg^{++} & 6.0 K^{+}	.58ab	1.17 cde	.44a	1.47 ef
0.5 Mg^{++} & 12.0 K^{+}	.86abcd	1.75 fg	.55a	1.78 fg
4.0 Mg^{++} & 12.0 K^{+}	.50a	1.08 cde	.42a	1.33 def
		per cent K^{+}		
0.5 Mg^{++} & 6.0 K^{+}	9.16 bc	12.50 d	6.97a	9.58 b
4.0 Mg^{++} & 6.0 K^{+}	8.95 bc	11.94 d	7.50a	9.82 c
0.5 Mg^{++} & 12.0 K^{+}	9.37 bc	12.77 d	7.28a	11.70 d
4.0 Mg^{++} & 12.0 K^{+}	9.82 c	12.22 d	8.15ab	11.70 d

Means within a cation not followed by the same letter are significantly different at the 5 per cent level.

Table 8a. - The effect of Mg^{++} level on the cation content of spinach

Mg^{++} Level	Mg^{++}	Ca^{++}	K^{+}
(meq/L)		per cent	
0.5	.32a	1.43 b	9.92a
4.0	1.08 b	.87a	10.01a

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 8b. - The effect of K^{+} level on the cation content of spinach

K^{+} Level	Mg^{++}	Ca^{++}	K^{+}
(meq/L)		per cent	
6.0	.70a	1.27 b	9.55a
12.0	.69a	1.03a	10.38 b

Means in a column not followed by the same letter are significantly different at the 5 per cent level.

Table 8c. - The effect of age of tissue on the cation content of spinach

Age	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Young	.42a	.64a	8.40a
Mature	.84 b	1.67 b	11.53 b

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 8d. - The effect of foliar leaching on the cation content of spinach

Treatment	Mg^{++}	Ca^{++}	K^{++}
		per cent	
Control	.73 b	1.13a	10.84 b
Leached	.66a	1.18a	9.09a

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 8e. - The effect of Mg^{++} level and age of tissue on the Mg^{++} content of spinach

Mg^{++} Level	Young	Mature
(meq/L)	per cent Mg^{++}	
0.5	.25a	.39 b
4.0	.59 c	1.56 d

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 8f. - The effect of K^{+} level and leaching on the Mg^{++} content of spinach

K^{+} Level	Control	Leached
(meq/L)	per cent Mg^{++}	
6.0	.79 c	.62a
12.0	.67ab	.71 bc

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 8g. - The effects of Mg^{++} level, K^{+} level, and foliar leaching on the Mg^{++} content of spinach

Mg^{++} Level	6.0 meq/L K^{+} Control	6.0 meq/L K^{+} Leached	12.0 meq/L K^{+} Control	12.0 meq/L K^{+} Leached
(meq/L)	per cent Mg^{++}			
0.5	.37a	.27a	.35a	.28a
4.0	1.20 c	.97 b	.99 b	1.14 c

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 9 contains the cation analysis of an experiment investigating the effects of Mg^{++} level, K^{+} level, age of tissue, Mg^{++} removal from the culture media, and foliar leaching on the cation content of Savoy Supreme spinach. Increasing the Mg^{++} level increased the Mg^{++} content of all tissues regardless of age (Table 9f) while decreasing the Ca^{++} content and significantly increasing the K^{+} percentage (Table 9a). Increasing the K^{+} level of the nutrient solution increased the K^{+} content and decreased the Ca^{++} percentage while the Mg^{++} content was only slightly depressed (Table 9b). Cation content increased with maturity (Table 9c). When Mg^{++} was removed from the culture media 4 days before harvest the Mg^{++} content was depressed and was accompanied by an insignificant increase in the content of the other cations (Table 9d). On the other hand, foliar leaching reduced the content of all cations (Table 9e). Both Mg^{++} removal and leaching produced significant decreases in the Mg^{++} content at the high Mg^{++} levels (Tables 9g and 9h). The results in Table 9i show that Mg^{++} removal depresses the Mg^{++} content only in mature leaves, however, at the high Mg^{++} level (Table 9j) all tissues are affected. Foliar leaching reduced the Mg^{++} content in all tissues (Table 9k).

Table 9. - The effects of Mg^{++} level, K^{+} level, age of tissue, Mg^{++} removal from the culture media, and foliar leaching on the cation content of spinach var. Savoy Supreme

	Nutrient Solution Composition (meq/L)							
	0.5 Mg^{++} & 6.0 K^{+}		4.0 Mg^{++} & 6.0 K^{+}		0.5 Mg^{++} & 12.0 K^{+}		4.0 Mg^{++} & 12.0 K^{+}	
Age of Tissue	Young	Mature	Young	Mature	Young	Mature	Young	Mature
Treatment	per cent Mg^{++}							
Control	.14a	.21abc	.39 e	1.44 j	.14a	.18ab	.36 de	1.29 i
Leached	.14a	.19ab	.26 bc	1.24 hi	.14a	.20abc	.26 bcde	1.22 hi
Mg-removed	.12a	.18ab	.27 bcd	1.16 gh	.14a	.18ab	.30 c	1.10 g
Mg-removed + Leached	.14a	.18ab	.18ab	.93 f	.12a	.13a	.21abc	.94 f
	per cent Ca^{++}							
Control	.81 cde	3.33 k	.72 bcde	2.78 j	.70 bcde	2.67 ij	.42ab	1.50 f
Leached	.45abc	2.36 hi	.33ab	1.76 fg	.33ab	2.11 h	.28a	1.53 f
Mg-removed	.89 de	3.67 k	.86 cde	2.20 h	.80 cde	2.67 ij	.95 e	2.06 gh
Mg-removed + Leached	.47abc	2.58 ij	.39ab	1.53 f	.44ab	2.03 gh	.53abcd	1.70 fg
	per cent K^{+}							
Control	6.16abc	9.37 efgh	6.48abc	8.54 ef	8.05 de	11.17 ijk	7.39 cd	11.21 jk
Leached	5.77ab	8.95 efgh	5.86ab	8.74 efg	6.46abc	9.99ghij	6.48abc	8.92efgh
Mg-removed	6.88 bcd	10.27 hij	7.07 bcd	9.58 fgh	7.18 cd	11.83 k	8.19 def	11.70 k
Mg-removed + Leached	5.69ab	9.02 efgh	5.34a	8.81 efg	6.46abc	9.02efgh	6.02abc	9.84 ghi

Means within a cation not followed by the same letter are significantly different at the 5 per cent level.

Table 9a. - The effect of Mg^{++} level on the cation content of spinach

Mg^{++} Level	Mg^{++}	Ca^{++}	K^{+}
(meq/L)		per cent	
0.5	.16a	1.64 b	8.27a
4.0	.73 b	1.22a	8.70 b

Means in a column not followed by the same letter are significantly different at the 5 per cent level.

Table 9b. - The effect of K^{+} level on the cation content of spinach

K^{+} Level	Mg^{++}	Ca^{++}	K^{+}
(meq/L)		per cent	
6.0	.45a	1.57 b	7.66a
12.0	.43a	1.29a	8.74 b

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 9c. - The effect of age of tissue on the cation content of spinach

Age	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Young	.21a	.58a	6.59a
Mature	.68 b	2.28 b	9.81 b

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 9d. - The effect of the removal of Mg^{++} 4 days before harvest from the culture medium on the cation content of spinach

Treatment	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Control	.49 b	1.38a	8.10a
Mg-removed	.45a	1.48a	8.30a

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 9e. - The effect of foliar leaching on the cation content of spinach

Treatment	Mg^{++}	Ca^{++}	K^{+}
		per cent	
Control	.48 b	1.69 b	8.82 b
Leached	.41a	1.18a	7.58a

Means within a column not followed by the same letter are significantly different at the 5 per cent level.

Table 9f. - The effect of Mg^{++} level and age of tissue on the Mg^{++} content of spinach

Mg^{++} Level	Young	Mature
(meq/L)	per cent Mg^{++}	
0.5	.14a	.18 b
4.0	.28 c	1.17 d

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 9g. - The effect of Mg^{++} level and the removal of Mg^{++} from the culture medium on the Mg^{++} content of spinach

Mg^{++} Level	Control	Mg-removed
(meq/L)	per cent Mg^{++}	
0.5	.17a	.15a
4.0	.81 c	.64 b

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 9h. - The effect of Mg^{++} level and leaching on the Mg^{++} content of spinach

Mg^{++} Level	Control	Leached
(meq/L)	per cent Mg^{++}	
0.5	.16a	.15a
4.0	.79 c	.66 b

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 9i. - The effects of age of tissue and removal of Mg^{++} from the culture medium on Mg^{++} content of spinach

Treatment	Young	Mature
	per cent Mg^{++}	
Control	.23a	.75 c
Mg-removed	.19a	.60 b

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 9j. - The effect of Mg^{++} level, age of tissue and removal of Mg^{++} from the culture media on the Mg^{++} content of spinach

	Control		Mg-removed	
Mg^{++} Level	Young	Mature	Young	Mature
(meq/L)	per cent Mg^{++}			
0.5	.14ab	.19 b	.13a	.19 b
4.0	.32 d	1.31 f	.25 c	1.04 e

Means not followed by the same letter are significantly different at the 5 per cent level.

Table 9k. - The effect of age of tissue and foliar leaching on the Mg^{++} content of spinach

Treatment	Young	Mature
	per cent Mg^{++}	
Control	.24 b	.72 d
Leached	.18a	.63 c

Means not followed by the same letter are significantly different at the 5 per cent level.

Experiments were designed to determine the role of the isolated cuticle in controlling the absorption and leaching of Mg^{++}. The data in Table 10 indicate preferential penetration of Mg^{++} through isolated spinach cuticles. Influx is consistently greater than efflux but not significantly. However the lower cuticle has a significantly greater permeability to Mg^{++} in both influx and efflux. The ratio of lower cuticle penetration to upper cuticle penetration is approximately 2:1 which agrees with the observed ratio of stomata.

Table 10. - The movement of Mg^{++} through isolated spinach cuticle

	Influx	Efflux
	$\mu g \times 10^{-2}/cm^2/hr$	
Lower cuticle	6.1 c	5.3 bc
Upper cuticle	3.6ab	2.4a

Means not followed by the same letter are significantly different at the 5 per cent level.

DISCUSSION

The results of the field experiment exemplify the cation balance theory and emphasize the importance of cation balance as a concept in soil fertility studies. It is recognized that the regimes employed were not optimum for plant growth and that the adversity of the conditions is reflected in the results. Nevertheless, a reasonable explanation can be applied to the observations as they are similar to the findings of Albrecht and Schroeder (1). They found a higher absorption of Mg^{++} and a more direct response of Mg^{++} content to treatments at a lower pH. The results of this experiment show the direct response to Mg^{++} treatment but lack the greater total absorption because of the otherwise poor soil conditions. The treatment response is probably due to the high availability of the treatment Mg^{++} at the low pH. The cation-balance theory developed by Shear, et. al. (34) is illustrated by the reduced Ca^{++} content. As the Mg^{++} content of the plant increases the Ca^{++} content decreases proportionately. When hydrated lime was added to the soil to raise the pH 220 lbs. Mg^{++}/acre was added also. It is believed that this amount of Mg^{++} masked all treatment effects at the higher pH until an extremely large quantity of treatment Mg^{++} was applied. The Ca^{++} content is also stable at the higher pH.

The sampling experiment validates the choice of the mature lamina sample for indicating the Mg^{++} content of the plant. In young tissue and when the Mg^{++} supply is low there is a uniform distribution within the plant. The young tissue is low in Mg^{++} because the metabolic functions requiring large amounts of Mg^{++} have not yet attained their maximum rates. The uniform distribution at low levels is due to the high mobility of the Mg ion within the plant. With increasing maturity the leaf lamina attains a position of dominance in the metabolism of the leaf. The requisite Mg^{++} is increased and it is transported to this area of high activity. This metabolic requirement accounts for the increasing accumulation of cation with age and the higher accumulation in the blade with comparison to the petiole. At the higher Mg^{++} levels the petioles would become somewhat unresponsive to a change in Mg^{++} level because Mg^{++} would not tend to accumulate in as great a quantity due to the lower metabolic activity. Therefore, at higher Mg^{++} levels inclusion of the petiole in the sample would only dilute the cation content since it has a significantly lower concentration at higher Mg^{++} levels than the leaf lamina. For this reason petioles were eliminated from the tissue sample. The situation regarding Ca^{++} and K^{+} accumulation was considerably different since they did accumulate in the petioles. This accumulation can be related to the reduced mobility of Ca^{++} and the apparent lack of a direct relationship with metabolism of both Ca^{++} and K^{+}.

Very low levels of Mg^{++} were used to determine the Mg^{++} content at which a Mg^{++} deficiency chlorosis would appear in a marketable, mature spinach leaf. By combining visual observation and cation analyses it can be determined that at below 0.15 per cent Mg^{++} in the mature leaves of Dixie Market spinach a chlorosis occurs. After several days characteristic necrotic "sunscald" lesions typical of Mg^{++} deficiency appear on the spinach foliage (3). The narrow range of the Mg^{++} levels limits the significance of the response due to increasing Mg^{++} levels, however, a significant response occurs as the critical level is reached. In this experiment Ca^{++} content did not decrease with increasing Mg^{++} levels because of the previous period in which the plants were grown on the complete nutrient solution. The plants accumulated high amounts of Ca^{++} while on the complete solution and the shorter period of low Mg^{++} level did not stimulate further Ca^{++} uptake as it would have done had the Mg^{++} supply been continuously low. The effect of maturity is not so great in the cases of Mg^{++} and K^{+} content because of their relative mobility. On the other hand, Ca^{++} is immobile and here the effect of age is very great. The combined mobility of K^{+} and Mg^{++} and the close range of Mg^{++} levels accounts for the lack of interaction between Mg^{++} and age in this experiment.

Very slight differences in Mg^{++} accumulation occurred among the spinach cultivars tested. All were equally efficient in Mg^{++} accumulation at low Mg^{++} levels, however, at the higher concentrations of Mg^{++} two varieties did accumulate significantly less Mg^{++}. A more efficient use of Mg^{++} in these varieties probably accounts for the difference since all cultivars were equally prolific in yield. This finding does not suggest that all cultivars would respond equally to low Mg^{++} levels since many observations of varietal and species variation in cation content have been noted (28, 47).

One of the interesting new developments in mineral nutrition of field plants has been the finding by Tukey and co-workers (39) that all of the elements essential to plant growth could be removed from the foliage in significant amounts by leaching. This aspect was also considered in this study and as the results show, large losses of cations did occur with leaching. There are three possible explanations for these losses - a dilution caused by growth during the leaching period, the leachability of the spinach foliage, and the leaching technique used. It in inconceivable that the first explanation could account for a decrease in content of at least 20 per cent during a 4-day period. The plant would have to increase in size a similar amount in a very short time when it had already reached marketable maturity before the leaching treatment began. The second explanation can be used to explain a large portion of the losses as the spinach plant has a type of foliage that could be readily leached by Tukey's criteria (40). The plant is naturally succulent with large leaves that have thin cuticles. It also has a savoyed surface which, like a pubescent one, will retain water for extended periods of time. The third explanation is also of interest. The leaching technique used was to spray water on the foliage from above from a lawn irrigation hose. This method provided uniform water distribution but due to the large quantity of water used some ran through the crock leaching the sand media. The effect of leaching the culture media was minimized by draining the crocks each morning and supplying them with their respective nutrient solutions. With this correction it was felt that the experimental conditions might simulate those of a sandy soil during a heavy rainstorm. Under these conditions reduction of Mg^{++}, Ca^{++}, and K^{+} contents were approximately 20 per cent. It is interesting that Tukey and Amling (42) found similar reductions (approximately 20 per cent) in field grown blueberry and apple as compared to greenhouse-grown or protected plants and they attribute the reduction to leaching by rain and dew.

Although the effect of leaching the culture media was minimized it was felt that an accurate estimate of the magnitude of this loss should be obtained. There were two ways to approach this problem - prevent leaching of the sand culture or leach the culture media and not the foliage. Both methods were tried and the best results were obtained using the latter method. By leaching Mg^{++} from the sand a 10 per cent reduction in Mg^{++} content occurred. Comparison between tables, as is statistically possible, because no significant interaction occurred between these two factors, indicates there is a 10 per cent significant reduction from the foliage. The findings of Tukey (41) which indicate that losses are greater when the supply to the plant is maintained and that translocation rates are increased during leaching suggest that the quantities lost here may be minimal. Tukey (39) has found a loss of 9 per cent of the root absorbed Ca^{45} during a 24 hour period.

The ion antagonism between Mg^{++} and K^{+} in two spinach varieties was also studied. The results were the same in two similar experiments. K^{+} could depress the Mg^{++} uptake in mature tissue and at the higher levels of Mg^{++}. However, it did not depress the Mg^{++} content below the critical level, probably because a relatively low level of K^{+} was employed.

Since the leaching losses were so great it was thought that a study of Mg^{++} influx and efflux through the spinach leaf cuticle would be of interest. The experimental method and apparatus was developed by Yamada (49) and assumes that the composition of the membrane is not changed by the isolation procedure. Using these methods it was determined that influx dominated efflux in both the upper and lower cuticles. While the difference here is not significant it is consistent and significant differences have been obtained using this apparatus (49). The lack of significance is probably due to a slight modification of the methods. Yamada used radioactive material, diffusing it into a relatively large volume of distilled water. In this work the same concentration of $MgSO_4$ was used but the nonradioactive material diffused into a relatively small volume of water. Experimental evidence indicates that the permanent negative charges on the inner surface of the cuticle play the major role in governing the influx and efflux rates and are probably the cause of the difference between rates (27, 50). With this in mind it can be seen that changing the volume of the $MgSO_4$ solution might minimize the effect of these charge sites. In working with a small volume of $MgSO_4$ the charges would tie up a greater proportion of the Mg^{++} thereby decreasing the efflux rate.

The differences between the upper and lower cuticles support the theory that stomates are influential in foliar absorption. Penetration rates are $1\frac{1}{2}$ to 2 times greater in the lower cuticle where stomates are $1\frac{1}{2}$ to 2 times more frequent. This method provides no control over the opening or closure of the stomates, therefore this factor must be ignored. One variable which might be minimized by this method is that of cuticle composition. Others (20, 21) have obtained differences in stomatal frequency by using different plant species. It seems more likely that cuticle composition would vary to a greater extent among different plant species than between the upper and lower cuticles of the same leaf. Therefore, it is felt that any variation due to composition in this study is negligible.

SUMMARY

Studies were conducted to investigate the Mg^{++} nutrition of Spinacia oleracea and the following results obtained:

1.) Both foliar applications and soil treatments with $MgSO_4$ are effective in increasing the Mg^{++} content of the plant material.

2.) An increase in the Mg^{++} content produces a simultaneous decrease in Ca^{++} and K^+ content.

3.) Leaf lamina tissue provides the most accurate estimate of the Mg^{++} content of the plant.

4.) When the level of Mg^{++} in the mature leaf is below 0.15 per cent a chlorosis develops which subsequently becomes necrotic.

5.) All cultivars tested possessed the same ability of accumulate Mg^{++} at low Mg^{++} levels although differences did exist at higher Mg^{++} levels.

6.) Foliar leaching significantly reduces the Mg^{++}, Ca^{++}, and K^+ content of spinach leaves. Losses are greater from plants grown at high nutrient levels and also from older tissue.

7.) High K^+ levels can depress the Mg^{++} content of the plant.

8.) Mg^{++} influx is greater than efflux in isolated spinach leaf cuticles.

9.) Lower leaf cuticles have a higher Mg^{++} penetration rate than upper leaf cuticles, possibly because of a higher stomatal frequency.

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